



National Cryo-EM Training Program

Dwight V. Nissley, PhD - Director, Cancer Research Technology Program, FNLCR

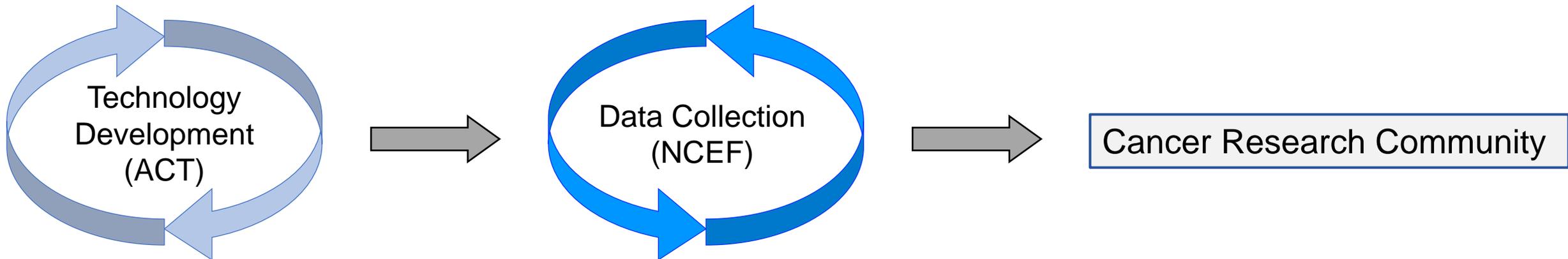
October 12, 2022

National Cryo-EM Facility 2017-present

Extramural user facility for cryo-EM data collection,
Ongoing expansion of scope, bandwidth and turn around

Cryo-EM Research and Development 2019-present

Newly created component to explore new platforms
Methods and technology development for cryo-EM field



Researcher with experience in cryo-EM technology

- have access to local screening microscopes
- need access to high-end instrumentation
- key drivers of growth of cryo-EM in the US

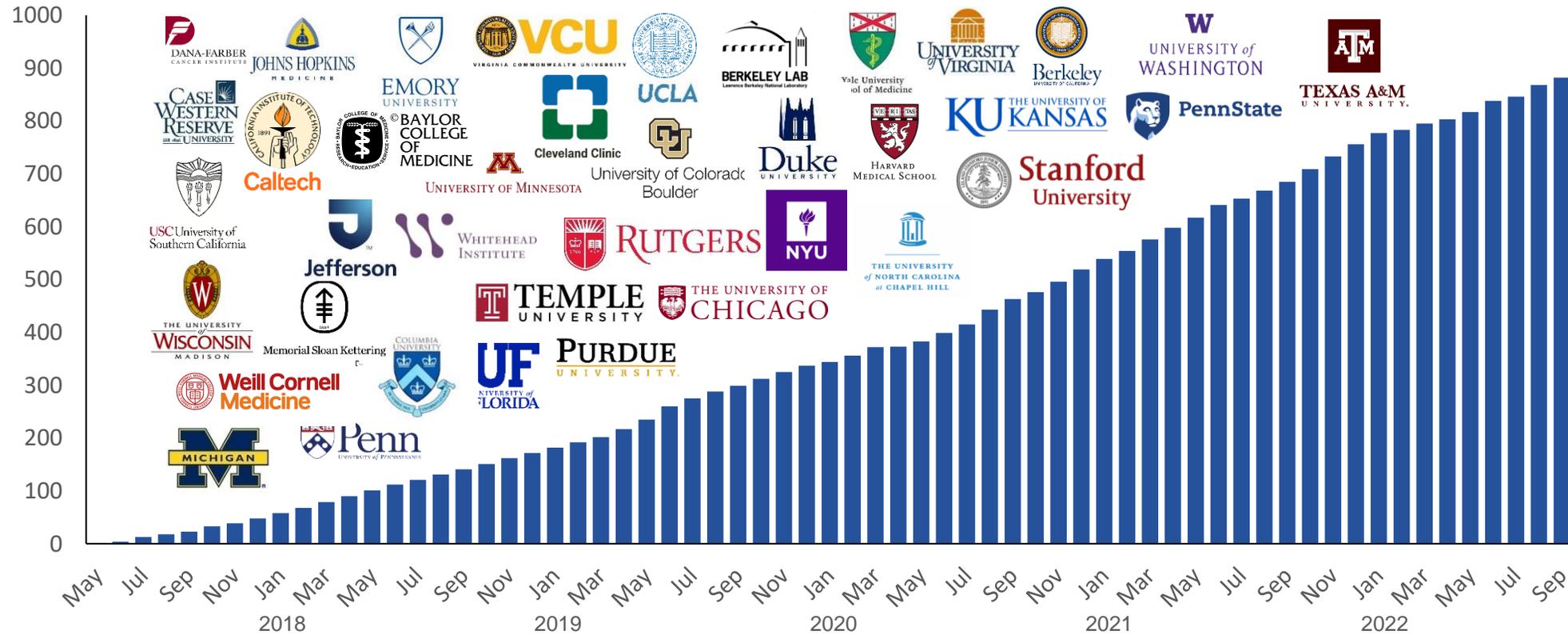
Structural biologists in adjacent disciplines (Crystallography, NMR)

- see value in using cryo-EM
- have expertise in protein biochemistry
- need training in cryo-EM specimen preparation, data collection, and processing

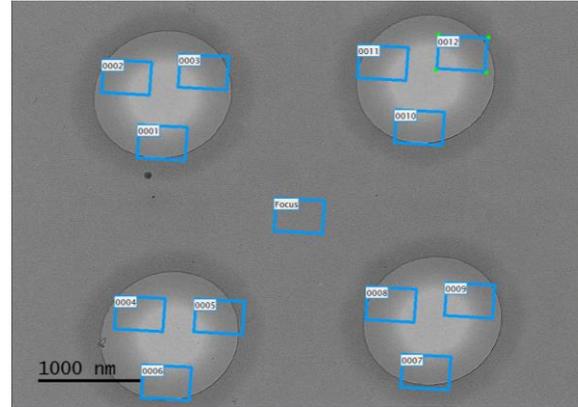
Biologists with interest in important biomedical problems

- interested in adding cryo-EM methods to their toolkit
- need training and collaboration in all aspects of the workflow from protein purification to the final interpretation of the structures

NCI National Cryo-EM Facility Operational Metrics



- 125 investigators from more than 50 institutions
- 880 imaging sessions completed.
- 80 publications in 5.5 years, with 29 publications in the past 12 months



Fringe Free Imaging

- multiple targets per hole
- more images from samples
- One microscope upgraded, other to be completed thus autumn

Two Titan Krios Microscopes

- Each is equipped with Gatan K3 Direct Detector and BioQuantum Energy Filter
- Current general imaging collects at 180 images/hour. 6000-7000 images for a two-day session
- New software and workflow, aim to double throughput

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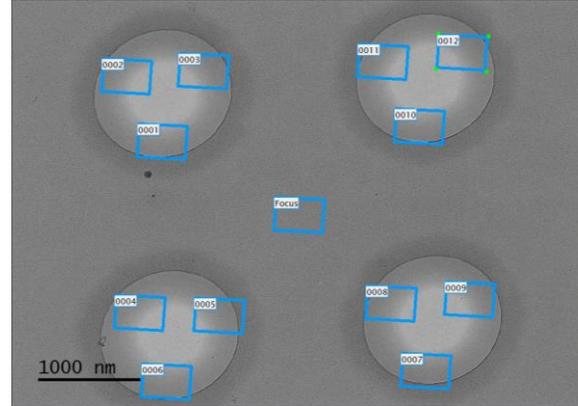
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NCEF Capabilities



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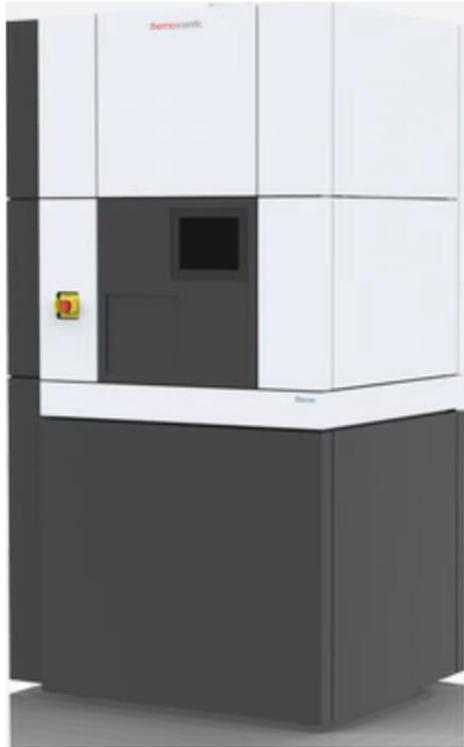
VitreJet

- Automated grid preparation
- Control and reproducibility
- Test with difficult sample
- Provide access to users

ACT Capabilities

Glacios

Thermo Fisher



electron source
X-FEG
200 kV



DE-64
Direct Electron



Falcon 3
Thermo Fisher

CryoARM 200

JEOL



electron source
Cold-FEG
200 kV

In-column **Omega**
energy filter



K3
GATAN Inc

Push resolution limits on lower-cost/voltage microscopes.

Broaden the potential to include inherently challenging samples.

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Cryo-EM Training Program

Overview

The inaugural NCEF Cryo-EM Training Program was held at the FNLCR's Advanced Technology Research Facility (ATRF) in Frederick, MD, September 12th through Friday, September 16th.

- Mornings featured guest lecturers (FNL experts and invited faculties) who provided extensive classroom learning on topics including sample preparation, grid screening, data collection and processing, structure determination and model building and validation.
- Afternoons moved into the National Cryo-EM Facility for comprehensive hands-on training in a laboratory setting.

FNL Technical Leads

- **Jana Ognjenovic, PhD**
- **Thomas Edwards, PhD**

Acknowledgements

- **Leonard P. Freedman, PhD**, Chief Science Officer, FNLCR
- **Crystal Canja & Vladimir Popov, PhD**, FNLCR Partnership Development Office
- **Gary Robinson, PhD**, NCI Office of Scientific Operations
- **Patti Martinez**, Cancer Research Technology Program, FNLCR

Cryo-EM Training Program – Attendees and Speakers

Cryo-EM Training Program Attendees

Name	Institution
Justin Cruite	Dana-Farber Cancer Institute
Albert Lau	Johns Hopkins University School of Medicine
Bibek Karki	University of Cincinnati
Fred Fregoso	University of Pennsylvania
Lakshmi Pidugu	University of Maryland School of Medicine
Rusia Lee	City University of New York (CUNY)
Stephanie Wankowicz	University of California San Francisco
Md Murad Khan	SUNY Upstate Medical University
Susmit Chaudhury	Los Alamos National Laboratory
Kyle McLaughlin	Medical College of Wisconsin
Loc Huynh	University of Florida
Lorenzo Dias Blattlin Briganti	University of Colorado

Cryo-EM Training Program Speakers

Name	Institution
Cynthia Wolberger	Johns Hopkins University
Ulrich Baxa	NIDDK
Thomas Edwards	FNL-NCEF
Giulia Wiessenberger	Cryosol-world
Ouliana Panova	SPTLabTech
Natalia de Val Alda	ThermoFisher
Bernard Heymann	FNL- ACT
Matt Hutchison	FNL-NCEF
Alex Noble	NYSBC
Tristan Croll	Altoslabs
Pavel Afonine	Lawrence Berkeley National Laboratory

Cryo-EM Training Program Agenda

Day 1

Keynote Address	Cynthia Wolberger , Ph.D., Johns Hopkins University
Negative Staining of Macromolecular Complexes	Ulrich Baxa , Ph.D., National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Grid Preparation	Thomas Edwards , Ph.D., Frederick National Laboratory for Cancer Research
Hands-on Training: Group 1- Vitrobot Grid Preparation , Group 2- Grid Clipping	Thomas Edwards, Jenny Wang, Adam Wier, Tara Fox , FNLCR

Day 2

VitroJet: Reproducibility and Control	Giulia Weissenberger , M.S., CryoSol-World
CryoEM Sample Preparation with Chameleon	Ouliana Panova , Ph.D., SPT Labtech
Hands-on Training: Group 1- Grid Clipping , Group 2- Vitrobot Grid Preparation	Thomas Edwards, Jenny Wang, Adam Wier, Tara Fox ; FNLCR

Day 3

The Electron Microscope: A Crash Course	Thomas Edwards , Ph.D., Frederick National Laboratory for Cancer Research
Advances in Electron Microscopy	Natalia DeVal Alda , Ph.D., Thermo Fisher Scientific
Managing CryoEM Data: Always Too Much and Nowhere to Put It	Matt Hutchison , Frederick National Laboratory for Cancer Research
Hands-on Training: Microscope Tutorial and Screening	Thomas Edwards and Tara Fox , FNLCR

Day 4

Image Formation in the Electron Microscope and Micrograph Processing for CryoEM	Bernard Heymann , Ph.D., Frederick National Laboratory for Cancer Research
Neural Network Particle Picking and Denoising with Topaz	Alex Noble , Ph.D., SEMC, New York Structural Biology Consortium (NYSBC)
Hands-on Training: Data Processing with CryoSparc	Joseph Finney, Matt Hutchison, Adam Wier, Jenny Wang , FNLCR; Alex Noble , NYSBC

Day 5

Physics-Based Interactive Model Building with ISOLDE	Tristan Croll , Ph.D., Altos Labs
Phenix for Cryo-EM	Pavel Afonine , Ph.D., Lawrence Berkeley National Laboratory
Hands-on Training: Modeling with Isolde and Phenix	Tristan Croll , Altos and Pavel Afonine , LBNL

National Cryo-EM Program

Dwight Nissley
Director, CRTP, FNLCR

National Cryo-EM Consultant
(Open)

- Recognized thought leader in cryo-EM
- Advise on emerging technologies
- Extramural cancer research community



Thomas Edwards
Senior Scientist
NCEF



Helen Wang
Program Manger
NCEF



Adam Weir
Microscopist
NCEF



Tara Fox
Microscopist
NCEF



Zhiqing Wang
Microscopist
NCEF



Matt Hutchison
IT Support
NCEF



Joseph Finney
IT Support
NCEF



Jana Ognjenovic
Senior Scientist
ACT



Alan Merk
Microscopist
ACT



Bernard Heymann
Biochemist
ACT



Dennis Winston
Biochemist
ACT

Cryo-EM Training Program Attendees

